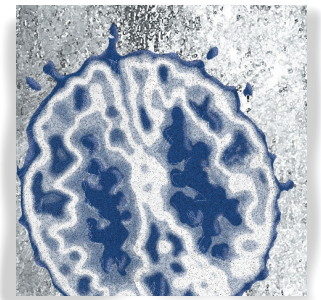
Mechanism-based treatments for Alzheimer's disease

Peter Davies, PhD; Jeremy Koppel, MD



Treatment for Alzheimer's disease is entering a new and exciting phase, with several new drugs beginning clinical trials. Many of these new therapies are based on our best current understanding of the pathogenesis of Alzheimer's disease, and are designed to try to either slow or halt the progression of the disease. There are several different theories underlying the current efforts, and these are briefly reviewed. Therapies directed against some aspect of β-amyloid formation, against neurofibrillary tangle formation and against the inflammatory response are all considered, as are the problems associated with each area. It is as yet unclear which, if any, of these approaches will be successful, but the high level of activity in each of these three fields provides some hope that an effective treatment for Alzheimer's disease is on the horizon.

© 2009, LLS SAS

Dialogues Clin Neurosci. 2009;11:159-169.

Keywords: *β-amyloid; secretase; aggregation; antibody; tau; protein kinase; inflammation*

Author affiliations: Litwin-Zucker Center for Research on Alzheimer's Disease, Feinstein Institute for Medical Research, Manhasset, NY, USA

A new era in therapy for Alzheimer's disease (AD) has begun, with several clinical trials putatively targeting the mechanisms fundamental to the disease process. At this point, however, there is still controversy as to which of the targeted processes truly are critical to disease progression, and how best to inhibit these. In this brief review, we will attempt to explain the molecular basis for the different therapies being tested, and to suggest where further knowledge is needed.

There are three different areas in which mechanism-based therapies have been developed: i) therapies targeting amyloid formation and/or deposition; ii) therapies targeting tau and/or neurofibrillary tangle formation; and iii) therapies targeting "neuroinflammation," or the gliosis accompanying formation of amyloid and tangle formation.

We will not consider therapeutic efforts that lack a clear molecular basis. While the discovery of an effective treatment does not always require information about the mechanism of the disease, rational translational research is greatly stimulated when molecular targets are preidentified.

Therapies targeting amyloid formation

The "amyloid cascade hypothesis"[1] has dominated translational research on Alzheimer's disease for over 20 years. As originally stated, this hypothesis placed emphasis on the deposition of β-amyloid as the initiating event in the neuronal dysfunction and death that occurs in brain.

Address for correspondence: Peter Davies, Litwin-Zucker Center for Research on Alzheimer's Disease, Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA
(e-mail: pdavies@nshs.edu)

Copyright © 2009 LLS SAS. All rights reserved

Selected abbreviations and acronyms

AD	*Alzheimer's disease*
APP	*amyloid precursor protein*
BACE1	*β secretase*
CDK	*cyclin-dependent kinase*
ERK	*extracellular signal-related kinase*
GSK	*glycogen synthase kinase*

Implicit in the arguments for this hypothesis is that excess production of β-amyloid occurs at some point in the disease process, although this has only rarely been demonstrated. The major arguments in favor of the hypothesis are genetic. Mutations in the gene encoding the precursor of β-amyloid (the amyloid precursor protein, or APP) are a very rare cause of familial Alzheimer's disease.[2] The most common causes of familial Alzheimer's disease are mutations in the presenilin 1 gene,[3] and presenilin 1 (as part of a multisubunit proteolytic enzyme called γ secretase) clearly plays an important role in cleavage of APP to produce β-amyloid.[4] Less common are mutations in the presenilin 2 gene,[5] and again this appears to function as part of a γ secretase complex. Thus all three genes in which mutation causes familial Alzheimer's disease are involved with proteolytic processing of the amyloid precursor protein.[6]

The discovery of amyloid deposits in both diffuse and neuritic plaques as a major characteristic of Alzheimer's disease pathology has been interpreted to mean that there is increased amyloid production. However, deposition could clearly be the result of decreased clearance, degradation, or of some other process occurring in the tissue. Recent data from three different groups has suggested that most of the familial Alzheimer's disease mutations in APP and presenilins 1 and 2 actually result in reductions in the rate of cleavage of the APP, and reduced rates of β-amyloid production.[7-9] This is clearly difficult to reconcile with the huge increase in amyloid deposits in brain tissue, and has led to modifications in the original pathogenic cascade model.

Indeed, over the last 10 years, more and more groups have moved away from the original formulation of the amyloid cascade hypothesis, in large measure because it is clear that there is only very limited neurotoxicity associated with deposition of β-amyloid. This is especially true in mice. A large number of transgenic mice have been made in which overexpression of mutant human APP (sometimes combined with a mutant presenilin1 gene) drives deposition of large amounts of β-amyloid in the brain. The vast majority of these transgenic mice do not have evidence of neuronal degeneration or cell death, nor do they feature neurofibrillary tangle formation. This result is not what would be expected if the original proposal of the amyloid cascade hypothesis were correct. These and other results have led to modifications of the original hypothesis that propose that it is not deposition of β-amyloid that is the initiating event in pathology, but the formation of a soluble "toxic species" of β-amyloid peptides.[10,11] Along this line of reasoning, some have suggested that the deposition of β-amyloid may in fact be neuroprotective,[12,13] with resultant sequestration of potentially toxic species. These toxic species are proposed to be oligomers, small aggregates of 2 to 12 peptide molecules, usually of the 42 amino acid long β-amyloid peptide.[11,14] There remains considerable controversy about the precise molecular nature of the toxic species, and about the mechanism by which this species produces detrimental effects on neurons. The most common explanation is that synaptic disruption is the immediate toxic event,[15] although precisely how this happens in the Alzheimer's disease brain remains poorly understood. Whether amyloid deposits or some soluble species is considered to be the initiating factor in the disease, these approaches are considered as "toxic gain of function models," in which disease is proposed to be caused by the formation of novel molecular entities that cause toxicity. There is now a fairly vocal minority of researchers who have proposed that it is not actually the formation of any β-amyloid species that is the problem. All of the known familial Alzheimer's disease mutations disrupt proteolytic processing of the amyloid precursor protein, and probably several other proteins normally cleaved by the γ secretase complex. If the production of a toxic β-amyloid species could be considered as a "toxic gain of function" in the majority view, the minority view would regard familial Alzheimer's disease mutations as "loss of γ secretase function." While this view would appear consistent with the apparent reductions in the rate of cleavage of the APP (and some other substrates) noted with mutant APP or presenilin 1, a major problem is to provide an explanation for the abundant deposition of β-amyloid in the Alzheimer brain. If less amyloid is made, why is there so much deposition?

Regardless of the position taken on the molecular details of APP processing in Alzheimer's disease, it remains true that the vast majority of attempts at therapy for Alzheimer's disease to date are directed at reducing the

amount of β-amyloid in brain. These attempts fall into four different groups, depending on the approach.

Use of inhibitors of amyloid aggregation

The first interventional amyloid approach, based on the unmodified amyloid cascade hypothesis, was an attempt to prevent amyloid aggregation and/or to disrupt preformed amyloid aggregates. Enthusiasm for this mechanism of intervention has waned somewhat, in tandem with the original version of the amyloid cascade hypothesis. Although a major clinical trial of an aggregation inhibitor, called Alzhemed[16,17] was carried out recently, results appear to have been negative, although some debate about variability between clinical trial sites has prevented a clear statement on this issue. Given the possibility that deposition of β-amyloid in tissues sequesters toxic species, and that disruption of deposition may increase toxic effects, further attempts along these lines appear unlikely.

Use of inhibitors of β-secretase

The proteolytic enzyme that cuts APP to liberate the N-terminus of the β-amyloid peptide, β secretase or BACE1, was identified and cloned by several groups, and it appears to be a single protein that cleaves APP and only a few other protein substrates.[18,19] Mice in which the *BACE1* gene is knocked out appear relatively normal, surviving into adulthood with subtle, if any, neuronal defects.[20] BACE1 appears to be essential for generation of β-amyloid, such that mice overexpressing mutant human APP do not generate any measurable β-amyloid in the absence of the mouse *BACE1* gene.[21] Clearly, the generation of specific inhibitors of BACE1 is an obvious and attractive prospect for prevention of production of β-amyloid. X-ray crystallography has been used to determine the precise structure of BACE1, and this should facilitate the development of inhibitors.[22] The nature of the active site of this enzyme presents significant challenges to the development of small molecule inhibitors that can cross the blood-brain barrier,[23,24] but it is very likely that such compounds will be forthcoming. Given the absence of a major detrimental effect of the knockout of the *BACE1* gene, inhibition of BACE1 appears unlikely to result in severe side effects (but see ref 25).
It is important to emphasize that success with BACE1 inhibitors will be dependent, to a large extent, on the validity of the "toxic gain of function" model, as suppression of BACE1 activity seems certain to reduce rates of production of β-amyloid by reducing rates of cleavage of APP. The challenge here is that if most mutations in APP and presenilin 1 also result in reduced rates of cleavage, and produce disease by this mechanism, one would expect an acceleration of disease progression on inhibition of either BACE1 (or γ secretase—see below). One of the most significant problems here is the absence of appropriate animal models. As mentioned above, mice with extensive amyloid deposition driven by overexpression of a mutant human APP gene do not develop a significant neurodegeneration. Thus while studies with BACE1 inhibitors could readily be performed in these mice to show reductions in amyloid deposition, few of the other features of Alzheimer's disease are evident in these mice, so that the effects of these compounds on the pathology and/or clinical features of Alzheimer's disease will remain untested until human trials are conducted.

Use of inhibitors of γ secretase

The problems with the use of γ secretase inhibitors are somewhat similar to those of inhibiting BACE1, although there are some notable distinctions. Knockout of vital components of γ secretase (presenilin 1, for example) does not produce viable mice unless the knockout is conditional[26] (effectively unless the knockout is engineered to occur only in adult mice). The problem here is that γ secretase cleaves numerous proteins as well as APP, and at least some of these proteins (eg, Notch1[27]) play critical roles in brain development. Their role in the adult animal is less clear, although knockout of both presenilins 1 and 2 in adult animals results in a striking neurodegeneration.[28,29] However, complete inhibition of γ secretase is not what is intended by therapeutics, and the question still remains about whether the production of β-amyloid can be reduced without unacceptable consequences, these resulting presumably from reductions in the rate of processing of other γ secretase substrates. Preliminary reports appear to suggest that this is possible,[30,31] and it appears that a large-scale phase 3 clinical trial of a γ secretase inhibitor is now underway. Again, success would seem to be dependent largely on the validity of the "toxic gain of function" model. There is perhaps the more direct concern here that again, the treatment exacerbates rather than interrupts the disease as reductions and not

increases in the activity of γ secretase appear to result from mutations, particularly in presenilin 1.

Finally, much has been made of the effects of mutations in presenilin 1 (and perhaps presenilin 2) on the ratio of β-amyloid 40 to β-amyloid 42 produced by APP cleavage.[7] These two peptides both appear to be produced by normal γ-secretase function, and it is true that many of the mutations shift the pattern of cleavage of APP so that despite the overall reduction in APP cleavage, relatively more of the 42 amino acid peptide is produced, decreasing the 40/42 ratio.[4] The β-amyloid 42 peptide does aggregate more readily than β-amyloid 40, and is more neurotoxic in in vitro assays.[32,33] The "toxic gain of function" model suggests that this is critical to the cascade of events that ensue. Precisely what inhibitors of γ secretase do to this ratio is unclear, although at least some published data indicates that suppression of γ secretase activity can occur without a significant change in the 40/42 ratio.[7] Perhaps this will prove critical to the success—or failure—of secretase inhibition in general. Only the clinical trials seem likely to provide this answer.

Use of antibodies, presumably to remove amyloid from the brain

Antibody approaches to reducing β amyloid in brain began with the spectacular studies of Schenk and colleagues, who immunized mutant human APP transgenic mice with β-amyloid peptides, and reported very significant reductions in amyloid deposition in these mice.[34] Several others have confirmed and extended this early work,[35,36] and human trials of "amyloid vaccination" have already been carried out. This is not the forum for discussing the controversial nature of these studies: suffice it to say that the results were far from the ideal. A number of patients developed an encephalitis,[37] and in some cases this appeared to be disastrous. Whether or not there was any benefit remains highly dubious,[38,39] but from a mechanistic viewpoint this approach raises a fundamental question: just how is an immune response to amyloid peptides supposed to reduce β amyloid concentrations in the brain?

Active immunization of transgenic mice with human amyloid peptides can produce the full range of B- and T-cell responses, in part because the human and mouse peptides differ in sequence—the human peptide is "foreign" to mice. Presumably the T-cell responses are what led to the encephalitis in humans immunized with human peptides, consistent with the induction of an autoimmune response.[36,40,41] But why would a B cell—an antibody-producing response—be helpful?

Generally, antibodies in the circulation penetrate into the brain in only low concentrations.[42] However, studies again in transgenic mice suggested that passive immunization, in which antibodies to β-amyloid were injected into the mice, have also been reported to cause significant reductions in the deposition of β-amyloid in the brains of the mice.[43,44] There are two basic ideas of how this might work. First, it seems possible that while only a very small fraction of the injected antibodies makes it across the blood-brain barrier, this is sufficient to bind enough β-amyloid to reduce deposition.[45,46] Antibody binding to β-amyloid in the brain may also activate the microglial (and possibly astrocytic) mechanisms that can reduce amyloid deposition.[44,47] Critical in this formulation is the penetration of antibody into the brain.

A second proposed mechanism is what has been called the "peripheral sink hypothesis." In this case, antibody binding to β-amyloid in the blood is thought to result in a sharp concentration gradient between the blood and the brain, such that β-amyloid movement from brain to blood is accelerated, and β-amyloid concentrations drop sharply and thus reduce the rate of deposition.[48] Although this mechanism initially seems highly unlikely, there is evidence for transport of β-amyloid from brain to blood, at least under some circumstances.[49] Perhaps it is unnecessary for the antibody to reach the brain at all.

The first clinical trials of "passive immunization" as a treatment for Alzheimer's disease appear to be underway, and preliminary results were reported in mid-2008. In passive immunization of transgenic mice, at least some antibodies appear to cause a shift in the localization of β-amyloid from deposits in the tissue to deposition in vessel walls, with some microhemorrhages reported.[43] Human trials reported some vasculitis as a side effect in groups receiving the highest doses of antibody, although effects on rates of cognitive decline did not appear to be large, if measurable at all. Further trials of passive immunization are underway, in some cases using intravenous immunoglobulin G (IgG) fractions, with the presumption that natural IgG fractions—prepared by isolation of IgG from many thousands of donors—contain sufficient concentrations of anti-β-amyloid antibodies to reduce amyloid deposition.[50,51] Whether this will prove a viable approach to therapy is as yet unclear.

Therapies targeting tau and/or neurofibrillary tangle formation

Tau, a microtubule-associated protein, is the major protein of neurofibrillary tangles. The amyloid cascade hypothesis considers that changes in tau leading to neurofibrillary tangle formation to be secondary events, and this viewpoint resulted in a neglect of this area in terms of therapeutics (with a few notable exceptions). A change in perception resulted from the discovery of mutations in the human tau gene that caused the neurodegenerative diseases collectively called frontotemporal dementia or tauopathies.[52] These diseases are characterized by massive degeneration of frontal and temporal cortex, frequently with Parkinsonian features and sometimes featuring extensive tangle pathology.[53] Since the initial reports, it has become clear that a number of single amino acid changes in tau result in neuronal degeneration, and that even mutations that do not alter the amino acid sequence can cause disease, by altering the splicing of the tau mRNA.[54] It appears that even quite small changes in tau can cause neuronal death (at least in the frontal and temporal cortex), and this notion has led to the hypothesis that the changes in tau seen in Alzheimer's disease may be responsible for cell death even if they are secondary to β-amyloid toxicity.[55,56] This hypothesis has received some support from experimental work in which β-amyloid has been shown to have less toxicity in cells or mice lacking tau.[57-59] Prevention of tau pathology has begun to emerge as a viable approach to prevention of neurodegeneration, although efforts in this area lag significantly behind the anti-amyloid research. There are currently two major approaches in this area: i) prevention of tau aggregation; and ii) inhibition of tau phosphorylation.

Prevention of tau aggregation

The major function of tau in neurons is thought to be the stabilization of microtubles, and tau can be demonstrated to both increase the rate of assembly of tubulin into microtubules and stabilize existing microtubules.[60] This activity appears to be controlled by phosphorylation of tau, in that phosphorylation of tau renders it less efficient in promoting assembly, and is believed to dissociate tau from assembled microtubules. Tau is ordinarily a soluble protein, but forms insoluble, filamentous aggregates in the course of neurofibrillary tangle formation. Early methods for purification of tangles took advantage of this insolubility, and employed harsh detergent and acid extraction techniques to dissolve away contaminating proteins.[61,62] Both toxic gain of function and loss of function models have been proposed for tau's role in neuronal degeneration. The formation of tau aggregates may be toxic to neurons,[63] or conversely, conversion of tau into insoluble polymers may reduce the effectiveness of tau in stabilizing microtubules. It is still too early in this research to decide which model is more plausible.

The mechanisms responsible for the conversion of a normally soluble monomeric protein into the insoluble filamentous aggregates have been the subject of intense study and the target for some drug development. Although tau in neurofibrillary tangles is hyperphosphorylated (see below),[64] there is still much debate about the role of phosphorylation in aggregation of tau. Indeed, many of the studies of tau aggregation have shown the formation of filamentous aggregates from nonphosphorylated tau,[65,66] and have used such systems to screen for potential inhibitors of tau aggregation. These studies usually include polyanions such as heparin, RNA,[65] or arachidonic acid[67] to stimulate tau aggregation. Using such a system, unpublished results apparently revealed that methylene blue could inhibit tau aggregation, and this compound, under the name Rember, has been reported to be effective in preventing decline in clinical test scores in patients with Alzheimer's disease. Abstracts claiming to describe the effects of Rember on tau aggregation in cell culture models, in tau transgenic mice, and even in human imaging studies have also appeared, although peer-reviewed data is not yet available to support these claims. Rember appears to be the first tau aggregation inhibitor to reach phase II clinical trials but whether or not the results reported can be replicated remains to be seen.

Prevention of tau phosphorylation

Tau in the adult human or animal brain is a phosphoprotein, with an average of about 2 moles of phosphate per mole of protein, while tau isolated from the Alzheimer brain (usually as neurofibrillary tangles) contains 6 to 8 moles of phosphate per mole of protein,[68] and there is thus little debate about the fact that tau is hyperphosphorylated.[69] The majority of the translational research on tau has centered on the development of drugs to inhibit this hyperphosphorylation, with the implicit assumption that

abnormal activation of a protein kinase activity is responsible for this increase in tau phosphorylation. Formally, it is possible that a deficiency in a protein phosphatase is as likely a culprit as an abnormal kinase activity, but it is usually easier to develop enzyme inhibitors than to develop enzyme activators. Careful analysis of the sites on tau at which phosphorylation is increased has suggested that these are the result of activation of more than one protein kinase.[70] This conclusion derives from work which identified hyperphosphorylation sites with a proline residue following a serine or threonine, this being required for the activity of the "proline-directed kinases,"[71] as well as phosphorylation at sites that lack a proline (where phosphorylation is presumably performed by other kinases). It appears probable that there is a cascade of kinase activities,[72] and the great difficulty has been in trying to identify a single critical kinase responsible for conversion of tau into neurofibrillary tangles.

Several protein kinases have been discussed as potential targets for therapeutics. Glycogen synthase kinase (GSK)3β, cyclin-dependent kinase (CDK)5 and extracellular signal-related kinase (ERK)2 seem to be the most commonly selected targets, and there has been at least some evidence published to suggest that all three kinases can be found associated with tangles in the brains of patients with Alzheimer's disease.[73] For GSK3β, it is well established that this kinase can phosphorylate tau in cells in culture[74] and in the brains of transgenic mice,[75] especially when a constitutively active kinase is used. There is also some evidence that GSK3β activity can accelerate the aggregation of tau when introduced into some tau transgenic mice,[76] although in certain mice the introduction of GSK3β actually resulted in less aggregation.[75,77]

A large number of inhibitors of GSK3β have been developed over the last few years, and two well-known agents, lithium and sodium valproate, are also thought to act at least in part through inhibition of this kinase.[78] The use of lithium with its attendant side effects and potential for toxicity poses a challenge to long-term use in demented elderly patients, and no substantial study of possible efficacy in Alzheimer's disease has yet been published (but see refs 79-82). Nonetheless, lithium, as well as some of newer synthetic GSK3 inhibitors, have been used in several tau transgenic drosophila[83,84] and mouse models, and there are reports of reductions in tau phosphorylation and aggregation.[85,86] Whether the newer inhibitors will make it into clinical trials is uncertain. GSK3 is involved in numerous cellular processes, including glycogen storage, control of cell division, and perhaps neuronal polarity. There is an obvious concern about the potential for side effects from inhibition of this kinase. Sodium valproate is apparently being tested in a large clinical trial in Alzheimer's disease patients[87] although no data are yet published.

Among the remaining candidate protein kinases, there is great interest in CDK5, the activity of which appears to play a very important role in brain development.[88] Transgenic mice in which CDK5 activity is activated (by overexpression of the p25 activator) in adult brain show evidence of a striking neurodegeneration with some tau pathology.[89,90] It has been reported that the concentration of p25 is elevated in the human AD brain,[91] although the validity of the original report has been questioned.[92] Inhibitors of CDK5 appear to have some influence on the development of pathology in some tau transgenic mice, although the effects reported are not large.[93] There are as yet no reports of the use of CDK5 inhibitors in humans.

Finally, activated ERK2 has been reported to be associated with neurofibrillary tangles in human Alzheimer's disease.[94] A rather nonspecific inhibitor of this kinase was used in tau transgenic mice, with apparently some beneficial results,[95] but as with GSK3 and CDK5, there are concerns that the multifaceted role of this kinase in cellular metabolism would appear to lower the probability that such inhibitors will make it into human studies.

One of the largest barriers to work of this type has been the lack of good research tools. Compounds that can specifically inhibit the activity of a single kinase (eg, GSK3β) and can efficiently cross the blood-brain barrier would allow better definition of the kinases that phosphorylate tau in vivo. There are few such compounds available to the research community. Precise definition of the kinases responsible for tau phosphorylation in the normal adult brain would be very helpful. As indicated above, there are a large number of different transgenic animal (and fly) models in which tau appears to be hyperphosphorylated, but it has been very difficult to dissect the signal transduction pathways responsible for this phosphorylation in any given model. There is, as always, also the concern about the fidelity of the models: how accurately do they reflect the process of tau hyperphosphorylation that occurs in human Alzheimer's disease?

Antibody approaches to tau pathology?

There is a single report in which a "vaccination" approach to the treatment of tau pathology was carried out in a tau

transgenic mouse model, with some apparent benefit.[96] It is hard to work out how this might work, as in contrast to the amyloid pathology, tau aggregates inside neurons. Are antibodies able to access abnormal tau within neurons? This seems very unlikely, but further studies along these lines appear to be going on in several transgenic mouse models. If this approach does produce promising results, it may prove difficult to unravel the mechanism by which this happens.

Therapies targeting "neuroinflammation"

The idea that the gliosis (microgliosis and astrocytosis, together called neuroinflammation) that accompanies the amyloid and tau pathology of Alzheimer's disease plays an active role in the neurodegenerative process has been much discussed over the last 15 years. Activated microglia, and perhaps activated astrocytes, can produce a variety of cytokines and other factors (especially reactive oxygen species, ROS) that in some circumstances appear to be neurotoxic. There is also evidence from epidemiological studies that chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs) was associated with a significant reduction in the risk for development of Alzheimer's disease.[97-99] Given the very widespread use of a number of different NSAIDs and other anti-inflammatory agents, a series of clinical trials were performed over the last decade. Despite some initial apparently positive effects in nonblinded studies, formal trials using prednisone,[100] rofecoxib,[101-103] naproxen,[104] celecoxib,[105] triflusa[106] and hydroxychloroquin[107] all yielded negative results. More recently, (R) flubiprophen, a derivitive of an NSAID that was also reported to have activity as a γ secretase inhibitor,[108,109] was reported to be without effect in a large clinical trial with several hundred patients with Alzheimer's disease. It is often easy to criticize a particular clinical trial for using only a limited number of doses of a few different compounds in a relatively small sample of patients. However, the results reported to date from studies testing potential anti-inflammatory drugs in patients with Alzheimer's disease are unanimous in their inconsistency with the idea that targeting this mechanism is likely to be fruitful. It remains possible that a better understanding of the relationship between the microgliosis/astrocytosis of Alzheimer's disease and classically defined peripheral inflammation would be worthwhile. As has been pointed out by others, the neuroinflammation of Alzheimer's disease is not classical inflammation, and the role of this response and the reaction to anti-inflammatory agents might be quite different.[110] Despite these caveats, it seems unlikely that additional clinical trials of agents of this type will be carried out in the new future.

Conclusions

We have briefly reviewed the approach of work aimed at developing mechanism-based therapies for Alzheimer's disease. Almost all of the individual areas could be the subject of a book, rather than a chapter or a section in a chapter. We have also only attempted to cover the major areas of research, and have left out several potentially very exciting areas that are at earlier stages of development. One such example is the "mitotic hypothesis," which suggests that much of the pathology in Alzheimer's disease results from inappropriate activation of cell cycle machinery in terminally differentiated neurons.[111-113] A recently published transgenic mouse study shows striking, Alzheimer-like degeneration from forced activation of the cell cycle with a viral oncogene.[114] The huge number of labs attempting to develop new agents for cancer treatment (antimitotics) may be expected to yield drugs that might be tested in such animal models, and perhaps in patients with Alzheimer's disease. We have also not discussed the very exciting area around apolipoprotein E (ApoE). There is now no doubt that a major risk factor for the development of Alzheimer's disease[115-119] (and perhaps other neurological diseases[120-122]) is the possession of one or more ApoE4 allele. Despite the wealth of evidence implicating ApoE4, there is as yet very little indication of a target for therapy in this area. It is to be hoped that all the basic research activity will change this situation soon.

We do not yet have truly effective therapy for Alzheimer's disease, but as the above review should make clear, there are several potential paths to such a treatment. It is our hope that all the activity in this area will soon yield real benefits to those who suffer from this dreadful disease. ❑

The authors' work is supported by NIMH38623, NIA022102 and NINDS048447, and by the Litwin-Zucker Center.

Tratamientos para la Enfermedad de Alzheimer según sus mecanismos patogénicos.

El tratamiento de la Enfermedad de Alzheimer está entrando en una nueva y excitante etapa, con algunos nuevos fármacos que están comenzando sus ensayos clínicos. Muchas de estas nuevas terapias se basan en la mejor comprensión actual de la patogenia de la Enfermedad de Alzheimer, y están diseñadas para intentar retardar o detener la progresión de la enfermedad. Se revisan brevemente diferentes teorías que están a la base de los esfuerzos terapéuticos actuales. Se consideran las terapias dirigidas contra ciertos aspectos de la formación de β-amiloide, contra la formación de ovillos neurofibrilares y contra la respuesta inflamatoria, así como los problemas asociados con cada una de las áreas. Aun no está claro si es que alguna de estas aproximaciones resultará exitosa, pero el alto nivel de actividad en cada uno de estos tres campos provee alguna esperanza de poder contar a futuro con algún tratamiento eficaz para la Enfermedad de Alzheimer.

Traitements de la maladie d'Alzheimer basés sur des mécanismes physiopathologiques

Le traitement de la maladie d'Alzheimer entre dans une nouvelle phase passionnante, des études cliniques permettant de lancer plusieurs nouvelles molécules. Nombre de ces nouveaux traitements sont basés sur la compréhension actuelle la plus poussée que nous ayons de la pathogenèse de cette maladie, et ils visent à ralentir ou arrêter son développement. Cet article passe brièvement en revue les différentes théories qui sous-tendent les efforts actuels. Les traitements dirigés contre certains aspects de la formation bêta-amyloïde, contre les dégénérescences neurofibrillaires et contre la réponse inflammatoire sont tous étudiés, de même que les problèmes associés à chaque localisation. Nous ne savons pas encore si une de ces approches aboutira, mais le niveau élevé d'activité dans ces trois domaines de recherche laisse espérer qu'un traitement efficace de la maladie d'Alzheimer se profile à l'horizon.

REFERENCES

1. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci*. 1991;12:383-388.
2. Hardy J. Molecular genetics of Alzheimer's disease. *Acta Neurol Scand Suppl*. 1996;165:13-17.
3. Sherrington R, Rogaev EI, Liang Y, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*. 1995;375:754-760.
4. De Strooper B, Saftig P, Craessaerts K, et al. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*. 1998;391:387-390.
5. Rogaev EI, Sherrington R, Rogaeva EA, et al. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature*. 1995;376:775-778.
6. Hutton M, Hardy J. The presenilins and Alzheimer's disease. *Hum Mol Genet*. 1997;6:1639-1646.
7. Bentahir M, Nyabi O, Verhamme J, et al. Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. *J Neurochem*. 2006;96:732-742.
8. Kumar-Singh S, Theuns J, Van Broeck B, et al. Mean age-of-onset of familial alzheimer disease caused by presenilin mutations correlates with both increased Abeta42 and decreased Abeta40. *Hum Mutat*. 2006;27:686-695.
9. Kakuda N, Funamoto S, Yagishita S, et al. Equimolar production of amyloid beta-protein and amyloid precursor protein intracellular domain from beta-carboxyl-terminal fragment by gamma-secretase. *J Biol Chem*. 2006;281:14776-14786.
10. Hardy J. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. *J Alzheimers Dis*. 2006;9(3 suppl):151-153.
11. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*. 2008;14:837-842.
12. Malm T, Ort M, Tahtivaara L, et al. beta-Amyloid infusion results in delayed and age-dependent learning deficits without role of inflammation or beta-amyloid deposits. *Proc Natl Acad Sci U S A*. 2006;103:8852-8857.
13. Winkler J, Connor DJ, Frautschy SA, et al. Lack of long-term effects after beta-amyloid protein injections in rat brain. *Neurobiol Aging*. 1994;15:601-607.
14. Walsh DM, Klyubin I, Shankar GM, et al. The role of cell-derived oligomers of Abeta in Alzheimer's disease and avenues for therapeutic intervention. *Biochem Soc Trans*. 2005;33(Pt 5):1087-1090.
15. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science*. 2002;298:789-791.
16. Aisen PS, Gauthier S, Vellas B, et al. Alzhemed: a potential treatment for Alzheimer's disease. *Curr Alzheimer Res*. 2007;4:473-478.
17. Aisen PS, Saumier D, Briand R, et al. A Phase II study targeting amyloid-beta with 3APS in mild-to-moderate Alzheimer disease. *Neurology*. 2006;67:1757-1763.
18. Vassar R, Bennett BD, Babu-Khan S, et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. 1999;286:735-741.
19. Haniu M, Denis P, Young Y, et al. Characterization of Alzheimer's beta -secretase protein BACE. A pepsin family member with unusual properties. *J Biol Chem*. 2000;275:21099-1106.
20. Roberds SL, Anderson J, Basi G, et al. BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. *Hum Mol Genet*. 2001;10:1317-1324.
21. Cai H, Wang Y, McCarthy D, et al. BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nat Neurosci*. 2001;4:233-234.
22. Moore KP, Zhu H, Rajapakse HA, et al. Strategies toward improving the brain penetration of macrocyclic tertiary carbinamine BACE-1 inhibitors. *Bioorg Med Chem Lett*. 2007;17:5831-5835.

23. Dominguez DI, Hartmann D, De Strooper B. BACE1 and presenilin: two unusual aspartyl proteases involved in Alzheimer's disease. *Neurodegener Dis*. 2004;1:168-174.
24. Lindsley SR, Moore KP, Rajapakse HA, et al. Design, synthesis, and SAR of macrocyclic tertiary carbinamine BACE-1 inhibitors. *Bioorg Med Chem Lett.* 2007;17:4057-4061.
25. Laird FM, Cai H, Savonenko AV, Farah MH, He K, Melnikova T, et al. BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *J Neurosci*. 2005;25:11693-11709.
26. Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell*. 1997;89:629-639.
27. Hartmann D, Tournoy J, Saftig P, Annaert W, De Strooper B. Implication of APP secretases in notch signaling. *J Mol Neurosci*. 2001;17:171-181.
28. Saura CA, Choi SY, Beglopoulos V, Malkani S, Zhang D, Shankaranarayana Rao BS, et al. Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. *Neuron*. 2004;42:23-36.
29. Shen J, Kelleher RJ, 3rd. The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. *Proc Natl Acad Sci U S A*. 2007;104:403-409.
30. Siemers ER, Quinn JF, Kaye J, et al. Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology*. 2006;66:602-604.
31. Fleisher AS, Raman R, Siemers ER, et al. Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. *Arch Neurol*. 2008;65:1031-1038.
32. Zou K, Kim D, Kakio A, Byun K, et al. Amyloid beta-protein (Abeta)1-40 protects neurons from damage induced by Abeta1-42 in culture and in rat brain. *J Neurochem*. 2003;87:609-619.
33. Irie K, Murakami K, Masuda Y, Met al. The toxic conformation of the 42-residue amyloid beta peptide and its relevance to oxidative stress in Alzheimer's disease. *Mini Rev Med Chem*. 2007;7:1001-1008.
34. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. 1999;400:173-177.
35. Schroeter S, Khan K, Barbour R, et al. Immunotherapy reduces vascular amyloid-beta in PDAPP mice. *J Neurosci*. 2008;28:6787-6793.
36. Buckwalter MS, Coleman BS, Buttini M, et al. Increased T cell recruitment to the CNS after amyloid beta 1-42 immunization in Alzheimer's mice overproducing transforming growth factor-beta 1. *J Neurosci*. 2006;26:11437-11441.
37. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med*. 2003;9:448-452.
38. Lee HG, Zhu X, Nunomura A, Perry G, Smith MA. Amyloid-beta vaccination: testing the amyloid hypothesis?: heads we win, tails you lose! *Am J Pathol*. 2006;169:738-739.
39. Ferrer I, Boada Rovira M, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol*. 2004;14:11-20.
40. Kim HD, Cao Y, Kong FK, Van Kampen KR, et al. Induction of a Th2 immune response by co-administration of recombinant adenovirus vectors encoding amyloid beta-protein and GM-CSF. *Vaccine*. 2005;23:2977-2986.
41. Grubeck-Loebenstein B, Blasko I, Marx FK, Trieb I. Immunization with beta-amyloid: could T-cell activation have a harmful effect? *Trends Neurosci*. 2000;23:114.
42. Seitz RJ, Heininger K, Schwendemann G, Toyka KV, Wechsler W. The mouse blood-brain barrier and blood-nerve barrier for IgG: a tracer study by use of the avidin-biotin system. *Acta Neuropathol*. 1985;68:15-21.
43. Wilcock DM, Rojiani A, Rosenthal A, et al. Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation*. 2004;1:24.
44. Wilcock DM, Rojiani A, Rosenthal A, et al. Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. *J Neurosci*. 2004;24:6144-6151.
45. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2001;98:8850-8855.
46. Bard F, Cannon C, Barbour R, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med*. 2000;6:916-19.
47. Morgan D. Modulation of microglial activation state following passive immunization in amyloid depositing transgenic mice. *Neurochem Int*. 2006;49:190-194.
48. Deane R, Sagare A, Hamm K, et al. IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. *J Neurosci*. 2005;25:11495-11503.
49. Bell RD, Sagare AP, Friedman AE, et al. Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. *J Cereb Blood Flow Metab*. 2007;27:909-918.
50. Kellner A, Matschke J, Bernreuther C, Moch H, Ferrer I, Glatzel M. Autoantibodies against beta-amyloid are common in Alzheimer's disease and help control plaque burden. *Ann Neurol*. 2009;65:24-31.
51. Weksler ME, Relkin N, Turkenich R, LaRusse S, Zhou L, Szabo P. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol*. 2002;37:943-948.
52. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998;393:702-705.
53. Morris HR, Khan MN, Janssen JC, Brown JM, Perez-Tur J, Baker M, et al. The genetic and pathological classification of familial frontotemporal dementia. *Arch Neurol*. 2001;58:1813-1816.
54. Morris HR, Perez-Tur J, Janssen JC, et al. Mutation in the tau exon 10 splice site region in familial frontotemporal dementia. *Ann Neurol*. 1999;45:270-271.
55. Hardy J. The relationship between amyloid and tau. *J Mol Neurosci*. 2003;20:203-206.
56. Small SA, Duff K. Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. *Neuron*. 2008;60:534-542.
57. Liu T, Perry G, Chan HW, et al. Amyloid-beta-induced toxicity of primary neurons is dependent upon differentiation-associated increases in tau and cyclin-dependent kinase 5 expression. *J Neurochem*. 2004;88:554-563.
58. Roberson ED, Scearce-Levie K, Palop JJ, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science*. 2007;316:750-754.
59. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A. Tau is essential to beta -amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A*. 2002;99:6364-6369.
60. Trinczek B, Biernat J, Baumann K, Mandelkow EM, Mandelkow E. Domains of tau protein, differential phosphorylation, and dynamic instability of microtubules. *Mol Biol Cell*. 1995;6:1887-1902.
61. Greenberg SG, Davies P, Schein JD, Binder LI. Hydrofluoric acid-treated tau PHF proteins display the same biochemical properties as normal tau. *J Biol Chem*. 1992;267:564-569.
62. Greenberg SG, Davies P. A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. *Proc Natl Acad Sci U S A*. 1990;87:5827-5831.
63. Congdon EE, Duff KE. Is tau aggregation toxic or protective? *J Alzheimers Dis*. 2008;14:453-457.
64. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A.* 1986;83:4913-4917.
65. Barghorn S, Davies P, Mandelkow E. Tau paired helical filaments from Alzheimer's disease brain and assembled in vitro are based on beta-structure in the core domain. *Biochemistry*. 2004;43:1694-1703.
66. Wilson DM, Binder LI. Polymerization of microtubule-associated protein tau under near-physiological conditions. *J Biol Chem*. 1995;270:24306-24314.

67. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol*. 1997;150:2181-2195.
68. Ksiezak-Reding H, Liu WK, Yen SH. Phosphate analysis and dephosphorylation of modified tau associated with paired helical filaments. *Brain Res*. 1992;597:209-219.
69. Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron*. 1992;8:159-168.
70. Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci U S A*. 2001;98:6923-6928.
71. Pelech SL. Networking with proline-directed protein kinases implicated in tau phosphorylation. *Neurobiol Aging*. 1995;16:247-56; discussion 57-61.
72. Liu F, Liang Z, Shi J, et al. PKA modulates GSK-3beta- and cdk5-catalyzed phosphorylation of tau in site- and kinase-specific manners. *FEBS Lett*. 2006;580:6269-6274.
73. Imahori K, Uchida T. Physiology and pathology of tau protein kinases in relation to Alzheimer's disease. *J Biochem*. 1997;121:179-188.
74. Sperber BR, Leight S, Goedert M, Lee VM. Glycogen synthase kinase-3 beta phosphorylates tau protein at multiple sites in intact cells. *Neurosci Lett*. 1995;197:149-53.
75. Spittaels K, Van den Haute C, Van Dorpe J, et al. Glycogen synthase kinase-3beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice. *J Biol Chem*. 2000;275:41340-41349.
76. Muyllaert D, Terwel D, Borghgraef P, Devijver H, Dewachter I, Van Leuven F. Transgenic mouse models for Alzheimer's disease: the role of GSK-3B in combined amyloid and tau-pathology. *Rev Neurol (Paris)*. 2006;162:903-907.
77. Nuydens R, Van Den Kieboom G, Nolten C, et al. Coexpression of GSK-3beta corrects phenotypic aberrations of dorsal root ganglion cells, cultured from adult transgenic mice overexpressing human protein tau. *Neurobiol Dis*. 2002;9:38-48.
78. Avila J, Hernandez F. GSK-3 inhibitors for Alzheimer's disease. *Expert Rev Neurother*. 2007;7:1527-1533.
79. Nunes PV, Forlenza OV, Gattaz WF. Lithium and risk for Alzheimer's disease in elderly patients with bipolar disorder. *Br J Psychiatry*. 2007;190:359-360.
80. Molchan SE, Manji H, Chen G, et al. Effects of chronic lithium treatment on platelet PKC isozymes in Alzheimer's and elderly control subjects. *Neurosci Lett*. 1993;162:187-191.
81. Kelwala S, Pomara N, Stanley M, Sitaram N, Gershon S. Lithium-induced accentuation of extrapyramidal symptoms in individuals with Alzheimer's disease. *J Clin Psychiatry*. 1984;45:342-344.
82. Randels PM, Marco LA, Hanspal NS, Robinson B. Possible lithium-induced extrapyramidal effects in Alzheimer's disease. *Hillside J Clin Psychiatry*. 1984;6:117-721.
83. Steinhilb ML, Dias-Santagata D, Fulga TA, Felch DL, Feany MB. Tau phosphorylation sites work in concert to promote neurotoxicity in vivo. *Mol Biol Cell*. 2007;18:5060-5068.
84. Steinhilb ML, Dias-Santagata D, Mulkearns EE, et al. S/P and T/P phosphorylation is critical for tau neurotoxicity in Drosophila. *J Neurosci Res*. 2007;85:1271-1278.
85. Noble W, Planel E, Zehr C, et al. Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc Natl Acad Sci U S A*. 2005;102:6990-6995.
86. Rockenstein E, Torrance M, Adame A, et al. Neuroprotective effects of regulators of the glycogen synthase kinase-3beta signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. *J Neurosci*. 2007;27:1981-1991.
87. Tariot PN. Clinical trials of amyloid-based therapies for Alzheimer's disease. *CNS Spectr*. 2007;12(1 suppl 1):7-10.
88. Tsai LH, Lee MS, Cruz J. Cdk5, a therapeutic target for Alzheimer's disease? *Biochim Biophys Acta*. 2004;1697:137-142.
89. Cruz JC, Tsai LH. Cdk5 deregulation in the pathogenesis of Alzheimer's disease. *Trends Mol Med*. 2004;10:452-458.
90. Cruz JC, Tseng HC, Goldman JA, Shih H, Tsai LH. Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. *Neuron*. 2003;40:471-483.
91. Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, Tsai LH. Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature*. 1999;402:615-622.
92. Tandon A, Yu H, Wang L, Rogaeva E, et al. Brain levels of CDK5 activator p25 are not increased in Alzheimer's or other neurodegenerative diseases with neurofibrillary tangles. *J Neurochem*. 2003;86:572-581.
93. Noble W, Olm V, Takata K, et al. Cdk5 is a key factor in tau aggregation and tangle formation in vivo. *Neuron*. 2003;38:555-565.
94. Roder HM, Hutton ML. Microtubule-associated protein tau as a therapeutic target in neurodegenerative disease. *Expert Opin Ther Targets*. 2007;11:435-442.
95. Le Corre S, Klafki HW, Plesnila N, et al. An inhibitor of tau hyperphosphorylation prevents severe motor impairments in tau transgenic mice. *Proc Natl Acad Sci U S A*. 2006;103:9673-9678.
96. Sigurdsson EM. Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies. *J Alzheimers Dis*. 2008;15:157-168.
97. McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology*. 1996;47:425-432.
98. Breitner JC, Gau BA, Welsh KA, et al. Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study. *Neurology*. 1994;44:227-232.
99. Breitner JC, Welsh KA, Helms MJ, et al. Delayed onset of Alzheimer's disease with nonsteroidal anti-inflammatory and histamine H2 blocking drugs. *Neurobiol Aging*. 1995 Jul-Aug;16:523-530.
100. Aisen PS, Davis KL, Berg JD, et al. A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology*. 2000;54:588-593.
101. Thal LJ, Ferris SH, Kirby L, et al. A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment. *Neuropsychopharmacology*. 2005;30:1204-1215.
102. Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *JAMA*. 2003;289:2819-2826.
103. Aisen PS, Thal LJ, Ferris SH, et al. Rofecoxib in patients with mild cognitive impairment: further analyses of data from a randomized, double-blind, trial. *Curr Alzheimer Res*. 2008;5:73-82.
104. Martin BK, Szekely C, Brandt J, et al. Cognitive function over time in the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT): results of a randomized, controlled trial of naproxen and celecoxib. *Arch Neurol*. 2008;65:896-905.
105. Group AR, Lyketsos CG, Breitner JC, et al. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. *Neurology*. 2007;68:1800-1808.
106. Gomez-Isla T, Blesa R, Boada M, et al. A randomized, double-blind, placebo controlled-trial of triflusal in mild cognitive impairment: the TRIMCI study. *Alzheimer Dis Assoc Disord*. 2008;22:21-29.
107. Van Gool WA, Weinstein HC, Scheltens P, Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. *Lancet*. 2001;358:455-460.
108. Weggen S, Eriksen JL, Das P, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature*. 2001;414:212-216.
109. Geerts H. Drug evaluation: (R)-flurbiprofen--an enantiomer of flurbiprofen for the treatment of Alzheimer's disease. *IDrugs*. 2007;10:121-133.
110. Szekely CA, Thorne JE, Zandi PP, et al. Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. *Neuroepidemiology*. 2004;23:159-169.
111. Herrup K, Yang Y. Pictures in molecular medicine: contemplating Alzheimer's disease as cancer: a loss of cell-cycle control. *Trends Mol Med*. 2001;7:527.
112. Vincent I, Pae CI, Hallows JL. The cell cycle and human neurodegenerative disease. *Prog Cell Cycle Res*. 2003;5:31-41.

113. Vincent I, Rosado M, Davies P. Mitotic mechanisms in Alzheimer's disease? *J Cell Biol*. 1996;132:413-425.
114. Park KH, Hallows JL, Chakrabarty P, Davies P, Vincent I. Conditional neuronal simian virus 40 T antigen expression induces Alzheimer-like tau and amyloid pathology in mice. *J Neurosci*. 2007;27:2969-2978.
115. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993;261:921-923.
116. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*. 1993;43:1467-1472.
117. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A*. 1993;90:1977-1981.
118. Roses AD. Apolipoprotein E affects the rate of Alzheimer disease expression: beta-amyloid burden is a secondary consequence dependent on APOE genotype and duration of disease. *J Neuropathol Exp Neurol*. 1994;53:429-437.
119. Roses AD, Saunders AM. APOE is a major susceptibility gene for Alzheimer's disease. *Curr Opin Biotechnol*. 1994;5:663-667.
120. Schmidt S, Barcellos LF, DeSombre K, et al. Association of polymorphisms in the apolipoprotein E region with susceptibility to and progression of multiple sclerosis. *Am J Hum Genet*. 2002;70:708-717.
121. Li YJ, Pericak-Vance MA, Haines JL, et al. Apolipoprotein E is associated with age at onset of amyotrophic lateral sclerosis. *Neurogenetics*. 2004;5:209-213.
122. Burwick RM, Ramsay PP, Haines JL, Hauser SL, Oksenberg JR, Pericak-Vance MA, et al. APOE epsilon variation in multiple sclerosis susceptibility and disease severity: some answers. *Neurology*. 2006;66:1373-1383.